Newborn Screening in Missouri
Program Overview and Test Addition

ABSTRACT

Missouri's Newborn Screening Program aims at early identification of infants who are affected by certain genetic or metabolic conditions. Early identification is essential as timely intervention can significantly reduce early morbidity and mortality and associated disabilities in these infants. The Newborn Screening Program is a collaborative effort of the Missouri Department of Health and Senior Services, genetic tertiary centers, physicians and other health professionals, and families. In accordance with national guidelines from the American College of Medical Genetics (ACMG), the Missouri Department of Health and Senior Services will add six amino acid disorders, eight fatty acid oxidation disorders and six organic acid disorders to the newborn screening panel beginning July 1, 2005. This article provides an overview of the Newborn Screening Program and describes the disorders that are detectable through the expanded screening. Technological advances have provided Missouri with yet another tool to improve the health of its citizens and prevent significant morbidity and mortality among its newborn babies.

OVERVIEW OF MISSOURI NEWBORN SCREENING AS A PUBLIC HEALTH PROGRAM

Newborn screening programs in the United States were the first population-based screening programs for genetic conditions and signaled the integration of genetic testing into public health programs. The mass screening of four million infants per year in the United States has been heralded as a successful program that is cost effective and reduces illnesses, disability, and death associated with inherited conditions. The universal acceptance of newborn screening for specified conditions since 1960 attests to the undeniable benefits that flow from testing and providing appropriate treatment and intervention.

Newborn screening has become widely recognized as a core public health program. Early diagnosis of disorders identified by newborn screening can lead to early treatment and prevent or minimize significant morbidity and mortality. The development of the Gutherie test for phenylketonuria (PKU) in 1963 and nationwide implementation of PKU screening programs by 1966 constituted landmark achievements in maternal and child health.

Newborn screening is a vital strategy in Missouri for preventing morbidity and mortality due to certain congenital disorders. The Missouri General Assembly passed initial newborn screening legislation in 1965. Through the years additional screens were added to the newborn screening panel. Currently, Missouri screens for the following disorders: phenylketonuria, congenital adrenal hyperplasia, congenital hypothyroidism, galactosemia and hemoglobinopathies. Missouri's Newborn Screening Program will add screening for six amino acid disorders, eight fatty acid oxidation disorders and six organic acid disorders beginning July 1, 2005.

Missouri law requires that all infants born in the state be screened for specified genetic disorders within the first 72 hours of life. Of the 75,530 Missouri live births reported in 2003, 99.9 % of the babies received newborn screening. This exceeded the Healthy People 2010 objective of 95% newborns screened.

Before an infant is discharged from his or her birth facility, a blood specimen is collected and sent to the State Public Health Laboratory for analysis. When the laboratory identifies an infant with an abnormal newborn screening result, the result is reported to the physician of record, the submitter (usually the birthing facility) and the Missouri Department of Health and Senior Services follow-up program. Abnormal results that fall into a "possible" to "high" risk or "presumptive positive" range are telephoned and faxed to the physician of record. If the baby is then diagnosed with a congenital disorder, the primary care physician and appropriate medical specialists collaborate to assure the infant receives necessary comprehensive care.

Fortunately, most babies have normal newborn screening results (87% in 2003). When an abnormal result is identified and reported to the infant's physician by the State Public Health Laboratory, the follow-up system is put into action. Follow-up activities can be relatively simple, such as the submission of a repeat specimen to the State Public Health Laboratory. In more complex cases, a team of individuals involved with the Newborn Screening Program coordinates efforts to obtain appropriate follow-up specimens.

The Missouri Department of Health and Senior Services is responsible for directing the Newborn Screening Program. In 1985, legislation created the Missouri Genetic Advisory Committee. The members of this committee are appointed by the Governor and meet at a minimum of once a year.

The committee is comprised of geneticists, specialty medical care providers, parents of affected children, primary medical care providers, genetic counselors, public health professionals and others involved in the identification and care of infants with the screened genetic disorders. All committee members generously volunteer their time and expertise. The committee advises the department on the provision of genetic services to ensure that: (1) high quality is maintained; (2) genetic programs are responsive to the needs of the entire state; (3) funding is equitably allocated to all phases of the program; (4) the department is advised on methods of implementing genetic services; (5) duplication of services is eliminated; and (6) a yearly evaluation of genetic programs is completed to ascertain how successfully the goals of the program are being achieved.

The Newborn Screening Standing Committee is a subcommittee of the Missouri Genetic Advisory Committee. The members of this committee include representatives from endocrinology, neonatalogy, and genetics; parents of affected children; and genetic counselors. All subcommittee members are volunteers. The subcommittee addresses issues of state and national concern such as establishing appropriate screening cutoffs to maximize sensitivity and specificity, developing protocols to ensure appropriate follow-up care after diagnosis and evaluating laboratory and clinical protocols to assure an efficient and beneficial program. The committee also addresses issues directly related to the conditions identified by the newborn screen and makes recommendations for the addition and deletion of a disorder to the newborn screen.

In 1999, the Newborn Screening Standing Committee began to consider adding additional screens to the newborn screening panel using tandem mass spectrometry (MS/MS) technology. This would enable the Newborn Screening Program to screen for amino acid disorders, fatty acid oxidation disorders and organic acid disorders. Missouri State law, RSMo 191.332, became effective in 2001, mandating the expansion of the newborn screening requirements "… to include potentially treatable or manageable disorders, including cystic fibrosis, galactosemia, biotinidase deficiency, congenital adrenal hyperplasia, maple syrup urine disease (MSUD) and other amino acid disorders, glucose-6-phosphate dehydrogenase deficiency (G-6-PD), MCAD and other fatty acid oxidation disorders, methylmalonic acidemia, propionic acidemia, isovaleric acidemia and glutaric acidemia Type I." Many of the conditions mandated by the legislation would be screened using tandem mass spectrometry. Beginning in 2004, the Department used one-time start-up funds to begin the process of expanding the newborn screening panel using MS/MS technology, including securing equipment and training staff in this new technology.

AMINO ACID DISORDERS

Amino acidopathies are caused by the body's inability to metabolize certain amino acids due to specific enzyme deficiencies. Buildup of amino acids and/or bi-products of amino acid metabolism in the blood causes severe medical complications. The presentation of the various amino acidopathies varies from no obvious clinical sequelae at birth (phenylketonuria), to acute encephalopathy (maple syrup urine disease, citrullinemia, arginosuccinic acidemia), within days following birth. In each of these disorders, the lack of early identification and treatment may result in serious medical consequences, including mental retardation, developmental delays, failure to thrive, and/or death. Because amino acidopathies are inherited in an autosomal recessive fashion, lack of family history is not an effective or efficient screening tool for these conditions.

Newborn screening by MS/MS allows for the identification of amino acidopathies by the measurement of amino acids. The amino acidopathies that Missouri will screen for using MS/MS technology include phenylketonuria (PKU), citrullinemia (ASS), arginosuccinic acidemia (ASA), homocystinuria (HCU), hypermethioninemia, maple syrup urine disease (MSUD), and tyrosinemia type II. Treatment of these disorders is accomplished with dietary restriction of the offending amino acid(s) and sometimes medications.

FATTY ACID OXIDATION DISORDERS

Fatty acid oxidation disorders occur secondarily to the body's inability to utilize and metabolize fatty acids for energy production. When the body is depleted of glucose, fatty acids are released for conversion into energy and ketones. The compound, carnitine, is responsible for transferring fatty acids into the mitochondria, where the bulk of fat metabolism occurs. After their transport, fatty acids are oxidized and broken down from long chain molecules into shorter chains and subsequently into energy in the form of ATP. Fatty acid oxidation disorders occur when fatty acid oxidation is hindered, due to either a specific enzyme deficiency in the fatty acid oxidation pathway, or a deficiency of carnitine. This leads to a buildup of toxic fatty acid metabolites, which may cause metabolic crisis and ultimately death.

The majority of fatty acid oxidation disorders become symptomatic when illness and concurrent fasting stress the patient. Affected individuals may appear well at birth but can develop life-threatening hypoglycemia following minor illness or periods of fasting. However, the stress of birth may be enough to induce illness. Medium chain acyl-CoA dehydrogenase deficiency (MCAD), is the most common fatty acid oxidation disorder, affecting approximately 1 in 10,000 individuals and has been found to account for up to 5% of the sudden infant death syndrome (SIDS) cases that occur. Children with MCAD typically appear normal at birth, but may develop hypoketotic hypoglycemia secondary to fasting, illness or excessive vomiting. Other symptoms include hypotonia, lethargy, intolerance to fasting, hypoglycemic coma, encephalopathy, hepatic failure or death. About 60% of affected individuals will die following their first metabolic crisis. Fatty acid oxidation disorders are inherited in an autosomal recessive manner, making the lack of family history of the condition an inefficient and ineffective screening tool.

The expansion of newborn screening by MS/MS allows screening for fatty acid oxidation disorders by the identification of disease specific acyl-carnitine patterns. Screening in Missouri will allow identification of infants with eight fatty acid oxidation disorders, including short chain acyl-CoA dehydrogenase deficiency (SCAD), medium chain acyl-CoA dehydrogenase deficiency (MCAD), long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD), trifunctional protein deficiency (TFP), very long chain acyl-CoA dehydrogenase deficiency (VLCAD), carnitine palmitoyl transferase deficiency types I and II (CPT I and CPT-II), carnitine/acylcarnitine translocase deficiency (CAT) and multiple acyl-CoA dehydrogenase deficiency (MAD). Screening by MS/MS may also allow the identification of other disorders of fat metabolism not listed above. Follow-up of an abnormal or positive newborn screen for a disorder of fatty acid oxidation metabolism may require submission of a repeat newborn screening card or more definitive testing such as urine organic acid analysis and quantitative acylcarnitine and urine acylglycine profiles.

Treatment of these disorders is accomplished by avoidance of fasting, supplementation with carnitine (in some of the disorders) and management of acute episodes with administration of IV glucose. Early diagnosis and treatment of these disorders allows proactive treatment and management to prevent or control metabolic crisis effectively.

ORGANIC ACID DISORDERS

Organic acidemias are a group of autosomal recessively inherited metabolic disorders that occur as a result of buildup of toxic organic acid intermediates due to the body's inability to break down certain amino acids and odd-chain fatty acids. Organic acidemias can cause life-threatening illness in early life resulting from metabolic crisis. Infants may present with an unusual odor, feeding problems, vomiting, lethargy, metabolic acidosis, ketosis or coma. Development may be affected due to hypotonia and failure to thrive. Because organic acidemias are inherited in an autosomal recessive fashion, lack of family history is not an effective or efficient screening tool for these conditions.

Newborn screening by tandem mass spectrometry allows for the identification of organic acidemias by identification of a specific pattern of acylcarnitines. The organic acidemias that Missouri will screen for with MS/MS technology include isovaleric acidemia (IVA), propionic acidemia (PA), glutaric acidemia type I (GA1), methylmalonic acidemia (MMA), 3-

methylcrotonyl-CoA carboxylase deficiency (3-MCC), and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG). Patients with these disorders are treated with a low-protein diet, and in some cases, supplementation with carnitine and/or vitamins. Early identification and treatment of patients with an organic acidemia can prevent recurring episodes of metabolic crisis.

Table 1. Clinical presentation and treatment of the amino acid disorders, fatty acid oxidation disorders and organic acid disorders included in the newborn screening panel.

Disorder	Clinical Presentation/Major Features	Treatment
Amino Acid Disorders		
Argininosuccinic aciduria (ASA)	Hyperammonemia, respiratory alkalosis, hyperventilation, lethargy, seizures, coma and death if not treated aggressively. Milder forms may present as developmental delays	Hemodialysis, L-arginine IV infusion and possible IV sodium benzoate and sodium phenylacatate acutely; low protein diet and oral arginine chronically
Citrullinemia (ASS)	Hyperammonemia, respiratory alkalosis, hyperventilation, lethargy, seizures, coma and death if not treated aggressively	Hemodialysis, IV L-arginine, sodium benzoate and sodium phenylacatate acutely; low protein diet, oral arginine and sodium phenylbutyrate chronically
Homocystinuria (HCU)	Developmental delay and mental retardation if untreated, increased risk for stroke and vascular disease, dislocated lenses of the eye, osteoporosis	Low protein diet, vitamin B6 (pyridoxine) Betaine
Hypermethioninemia	May be asymptomatic at birth; brain abnormalities including demyelination	Low protein diet
Maple syrup urine disease (MSUD)	Metabolic crisis, ketoacidosis, lethargy, poor feeding, respiratory distress, hypotonia alternating with hypertonia, coma, seizures, burnt sugar odor	Leucine, isoleucine and valine restricted diet, prompt treatment of crisis
Phenylketonuria (PKU)	No acute signs of illness in the newborn period, musty or mousy body odor, mental retardation if untreated	Low phenylalanine diet
Tyrosinemia, type II	Eye problems including photophobia, pain and redness due to corneal involvement, palmo-plantar keratosis, variable mental retardation	Low tyrosine and phenylalanine diet, vitamin B6 (pyridoxine)
Fatty Acid Oxidation Disorders		
Carnitine/acylcarnitine translocase deficiency (CAT)	Hypoketotic hypoglycemia, poor appetite, vomiting, lethargy, cardiomyopathy, cardiac arrhythmias	Avoid fasting and catabolic condition, high carbohydrate, low long-chain fatty acid (Portagen) with frequent feedings, supplement with medium chain triglycerides
Carnitine palmitoyl transferase deficiency (CPT)	Hypoketotic hypoglycemia, cardiomyopathy, hepatomuscular symptoms	Avoidance of fasting, prompt treatment of crisis
Long-chain hydroxyl acyl-CoA dehydrogenase deficiency (LCHAD)	Reye-like episode associated with hypotonia, hypoglycemia and hypertrophic cardiomyopathy	Low long chain fat formula, carnitine, medium chain triglyceride, prompt treatment of crisis
Medium-chain acyl-CoA dehydrogenase (MCAD)	Reye-like episode: vomiting, lethargy, encephalopathy, hepatomegaly, seizures, coma and death	Low fat, high carbohydrate diet, carnitine, prompt treatment of crisis

Multiple acyl-CoA dehydrogenase deficiency (MAD) (GA-II)	+/- major congenital anomalies, hypotonia, hypoglycemia, acidosis, sweaty foot odor, hepatomegaly	Low fat and low protein diet, carnitine, riboflavin, prompt treatment of crisis.
Short-chain acyl-CoA dehydrogenase deficiency (SCAD)	Metabolic crisis, encephalopathy, cardiomyopathy, failure to thrive, hypotonia, hepatomegaly	Low fat, high carbohydrate diet, carnitine, riboflavin, prompt treatment of crisis
Trifunctional protein deficiency (TFP)	Reye-like episode associated with hypotonia, hypoglycemia and hypertrophic cardiomyopathy	Low long chain fat formula, carnitine, medium chain triglyceride, prompt treatment of crisis
Very long-chain acyl-CoA dehyrogenase deficiency (VLCAD)	Metabolic crisis with vomiting, lethargy, hypotonia, cardiomegaly, encephalopathy, hepatic failure, coma and death	Low fat formula, carnitine, medium chain triglyceride, prompt treatment of crisis
Organic Acid Disorders		
Glutaric acidemia type I (GA-I)	Hypotonia, seizures, opisthotonos, loss of head control, tongue thrusting, dystonia, macrocephaly	Protein avoidance, carnitine, riboflavin, avoidance of fasting
3-hydroxy-3-methylglutaryl CoA lyase deficiency (HMG)	Reye-like episode: vomiting. Lethargy, coma, hypoglycemia, seizures	Low fat and protein diet, carnitine
3-methylcrotonyl CoA carboxylse deficiency (3-MCC)	Reye-like episode: vomiting, lethargy, apnea, hypotonia, seizures, coma	Leucine restricted diet, carnitine, avoidance of fasting
Isovaleric acidemia (IVA)	Ketoacidosis, vomiting, lethargy, coma, seizures, sweaty foot odor	Leucine restricted diet, carnitine, glycine, avoidance of fasting
Methylmalonic acidemia (MMA)	Metabolic crisis, ketoacidosis, lethargy, failure to thrive, dehydration, respiratory distress, hypotonia, coma, seizures	Low protein diet, carnitine, cobalamin, avoidance of fasting
Propionic acidemia (PA)	Metabolic crisis, ketoacidosis, lethargy, failure to thrive, dehydration, respiratory distress, hypotonia, coma, seizures	Low protein diet, carnitine, avoidance of fasting

THE ADDITION OF FATTY ACID OXIDATION DISORDERS, ORGANIC ACID DISORDERS AND AMINO ACID DISORDERS TO THE NEWBORN SCREENING PANEL

An extensive review by the Newborn Screening Standing Committee determined that these disorders meet the Newborn Screening Program's established criteria for the addition of a new screening test.

1. Incidence: The cumulative incidence of these disorders is estimated to be greater than 1/5,000 live births.

2. Morbidity and mortality: Given that each of these disorders can cause severe morbidity and/or mortality if untreated, they meet the criteria related to morbidity and mortality.

3. Potential for successful treatment: Effective treatment strategies exist for these disorders. The effectiveness of early dietary therapy in preventing morbidity and mortality has been well documented for MCAD and long-term prognosis is very good.[1] Likewise, dietary

and vitamin therapy for some of the organic acid disorders such as PA has also been shown to be effective.[2] Dietary therapy for other disorders, such as LCHAD, greatly improves many symptoms (i.e. hypoglycemia, hypotonia, hepatomegaly, cardiomyopathy and lactic acidosis) but not others (i.e. peripheral neuropathy, pigmentary retinopathy or myoglobinuria).[3]

4. Laboratory feasibility: Due to recent advances, appropriate technology is available to screen a large population for these disorders.

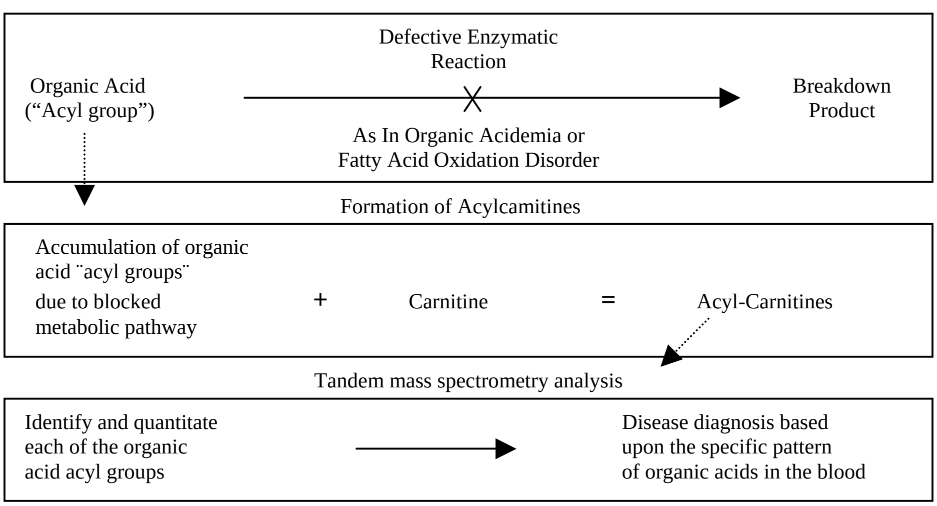
5. Costs: The cost per disorder detected is comparable to the other newborn screening tests currently in the screening panel.

NEW SCREENING TECHNOLOGY

The technology that has enabled screening for amino acid disorders, fatty acid oxidation disorders and organic acid disorders utilizes tandem mass spectrometry (MS/MS) to simultaneously measure more than 30 metabolic analytes at the rate of 90 seconds per sample. The test requires no additional blood specimen because it is performed on the standard filter card submitted for newborn screening, and testing for the additional 20 disorders will utilize the same blood that is currently being used just for phenylalanine screening.

Acylcarnitines are formed by esterification of organic acids to the endogenous amino acid, carnitine. Elevations in particular organic acid(s) in the blood, as occurs in organic acidemias and fatty acid oxidation disorders, will result in the elevation of corresponding acylcarnitine species. Each organic acidemia and fatty acid oxidation disorder has a characteristic acylcarnitine profile or pattern. The acylcarnitine profiles facilitate accurate diagnosis by identifying the accumulation of specific intermediates at a metabolic block. Figure 1 illustrates the block in the metabolic pathway. Screening for the amino acid disorders works in the same way in that it detects elevated amino acid levels due to blocks in their metabolic pathways.

Figure 1. Classical Illustration of Disrupted Metabolic Pathway



At the Missouri State Newborn Screening Laboratory, data from the tandem mass spectrometer are monitored by a dedicated computer that flags any specimen that has one or more amino acid or acylcarnitine above the normal cutoff. The analytical data from these samples are examined closely to determine if they match one of the profiles included in the screen. Those samples that meet the criteria for a given disorder are reported to the physician of record and to a consulting metabolic geneticist. A repeat newborn screen and other specialized tests, varying by disorder, are required to confirm or rule out a disorder.

Infants with a confirmed amino acid disorder, fatty acid oxidation disorder or organic acidemia disorder are referred to a genetic tertiary center. At the genetic tertiary center, families are offered medical, nutritional and genetic counseling services.

TANDEM MASS SPECTROMETRY SCREENING DURING THE PILOT PHASE

In late spring 2005, the Newborn Screening Program began the pilot phase for expanded newborn screening using MS/MS. The purpose of the pilot phase was to validate the tandem mass spectrometry testing system and to determine the normal/acceptable ranges for all of the analytical parameters that will be utilized in the screening process. This was achieved by using population data on each crucial metabolic analyte accumulated from more than 10,000 Missouri newborn blood samples. From this population data, the means, standard deviations, and ultimately the cutoffs, were determined for each of the metabolic analytes that were monitored in the screening process. It is important to understand that data generated during the pilot phase was not reported as an expanded screening result. In the event a result was flagged, which suggested a high risk of one of the disorders, arrangements were made to alert genetic consultants immediately, at one of the four genetic tertiary centers in Missouri, for follow-up.

This pilot phase will continue until full statewide implementation on July 1, 2005, at which time the Missouri Newborn Screening Program will commence expanded newborn screening.

SUMMARY

The addition of amino acid disorders, fatty acid oxidation disorders and organic acid disorders to Missouri's newborn screening panel is another example of a public health program working to improve the health and well-being of Missouri citizens. The success, as always, depends upon the excellent working relationship of all partners involved: the Missouri Department of Health and Senior Services, medical specialists, primary care physicians and families.

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The Missouri Department of Health and Senior Services would like to thank the WI Newborn Screening Program and acknowledge that much of the information for this article was obtained from "Newborn Screening in Wisconsin: Program Overview and Test Addition" printed in the Wisconsin Medical Journal. It was written by staff from the WI Newborn Screening Program, WI Division of Public Health; State Laboratory of Hygiene, University of Wisconsin-Madison; and Genzyme Genetics in Philadelphia. In addition, the following individuals also contributed to the writing and review of this article: Kathy Grange, M.D., geneticist at St. Louis Children's Hospital, Washington University, St. Louis, Mo.; Dawn Peck, MS, CGC, genetics counselor at the University Hospital and Clinics, University of Missouri-Columbia, Columbia, Mo.; and the staff of the Missouri Department of Health and Senior Services, State Public Health Laboratory, Newborn Screening Program and the Unit of Genetics and Newborn Health.